THE PREDOMINANCE OF COUNTERCLOCKWISE ROTATION DURING SWARMING OF BACILLUS SPECIES

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The tendency for growth or motion to follow a spiral path is very widespread in nature. Among the bacteria this is rather dramatically illustrated in the group movements exhibited by certain species that swarm over the surface of solid media. Because movement is confined to two dimensions in swarming, the phenomenon provides conditions that are suitable for a study of motion.

The swarming of species of *Proteus* has been observed in detail many times. Less well known are the peculiarities of swarming shown by some species of *Clostridium* (Turner and Eales, 1941*b*) and certain species of *Bacillus* (Muto, 1904; Henricksen and Svendsen, 1946; Roberts, 1935; Roszdestvensky and Medvinska, 1938; Russ-Münzer, 1938), notably *Bacillus circulans* (Smith and Clark, 1938; Turner and Eales, 1941*a*) and *Bacillus alvei* (Shinn, 1938; Smith and Clark, 1938; Shinn, 1940). Swarming among the *Eubacteriales* follows a general pattern (Murray and Elder, 1948). The extension over the agar surface is accomplished by groups of bacteria, moving as units. These units may take two forms: (1) "bullet-shaped" colonies of organisms moving at relatively high speed, almost always in curves, and (2) round colonies, rotating as plates on the surface of the medium, which may at the same time move in a direction. The bullet colonies seem to be common to all swarming species, but the rotating colonies are more highly developed in the swarming species of *Bacillus*, particularly *B. circulans*. The bullet colonies may travel in decreasing spirals until head meets tail and they become rotating colonies. Each type of colony leaves a trail of organisms from which originate new crops of swarming units.

In 1904 Muto described the general characteristics of swarming for an organism he named *Bacillus helixoides* (an organism that cannot be identified now although its general swarming characteristics resemble *B. circulans*). He noted that bullet colonies may become rotating colonies and that rotating colonies, originating from the track of another, do not necessarily rotate in the same direction as the "mother" colony. Other workers (Roberts, 1935; Russ-Münzer, 1938) have made similar observations since then. Russ-Münzer (1938) states: "The direction in which the colonies turn seems to be neither endogenous nor determined by the solidity of the agar—but to be determined by chance." Using *B. alvei*, Shinn in 1938 observed (using time-lapse cinephotography) that "of the probably 200–300 rotating colonies shown in the film, only two have been detected whose motion is clockwise." In 1940 Shinn made the statement that 95 per cent of the rotating colonies of *B. alvei* turned in a counterclockwise direction. Such a distribution would not be expected to occur by chance. Preliminary observations on this phenomenon have been reported in abstract by Murray and Elder (1949). In the present paper figures are presented which may be evaluated statistically.

METHODS

The swarming species of *Bacillus* used in this study were *B. circulans*, *B. alvei*, and *B. sphaericus* var. *rotans*. They were maintained and observed upon a papain broth medium (Asheshov, 1941) containing 1 per cent peptone (Difco) and 1.2 per cent agar (granulated, obtained from Agar Products, Ltd.). When fluid medium was used it consisted of the same medium without the agar. The organisms were grown and observed at room temperature.

Many of the observations were made by direct microscopy with a low-power objective (10 X) on the surface of agar plates. For some observations a modified form of "hanging block" preparation was used. For these preparations a thin layer of agar was flooded onto the surface of a cover glass, allowed to solidify, inoculated at a central point, and then inverted over a cell on a slide to which it was sealed. These preparations were used for observation with a 4-mm objective (45 X).

Directions of rotation or curvature are all expressed as if the observer were looking directly at the agar surface from above with the unaided eye.

OBSERVATIONS

The polarity of individual cells and swarming units. Observation of the vegetative cells of *B. circulans*, *B. alvei*, and *B. sphaericus* var. *rotans* in a fluid medium gave no hint of a constant polarity. When single cells were followed, some were observed to stop and frequently to restart in the reverse direction, without turning end for end. We must conclude, without any statistical evidence, that the individual cell has no constant polarity.

In swarming we are no longer dealing with the individual cell. The cells band together in groups, and each group has a polarity that is maintained until the group is disrupted by impact with a large mass or grows to a size restricting movement to zero. The principles that govern this aggregation into a swarming unit with polarity are unknown.

The relationship of swarming to motility. Those species among the *Eubacteriales* that exhibit swarming are all motile in fluid media and possess peritrichate flagella. The converse is not true, however, indicating some additional requirement for the ability to swarm. There is evidence (Boltjes, 1948) that swarming organisms have an extraordinarily large number of long flagella. This suggests that, in part, swarming may be a matter of motive power. Nonmotile variants occur (Clark, 1939) which have lost the ability to swarm.

With a technique suggested by Ørskov (1947), a thin layer of centrifuged India ink was placed in the path of swarming units and observed microscopically. Around the periphery of the moving unit it was seen that the particles of India Ink were agitated in a fashion suggesting flagellar activity.

If there is a direct relation between swarming and motility, substances that interfere with the swarming phenomenon should act on motility in fluid media. With Congo red and dahlia violet (previously used to prevent swarming), bromthymol blue (accidentally found to inhibit swarming), sorbitan monooleate and "tergitol 4" (both surface-active agents) it was found that motility in fluid medium and swarming on solid medium were inhibited at the same concentration levels in each case.

We may conclude, therefore, that the motive power for swarming is provided by the same mechanism that propels individual organisms in fluid media; this mechanism is probably provided by the flagella, despite the contentions of Pijper (1946), which have been ably rebutted by Boltjes (1948) and others.

The direction of movement of swarming units. If it is assumed that the movement of individual cells in a fluid is at random, it would be expected that the movement of swarming units in their two-dimensional field also would be at random. On casual inspection this appears to be true, because a plate inoculated at a point becomes covered in a random fashion by the swarm. However, as has been stated, Shinn (1940) observed that the majority of rotating colonies of *B. alvei* revolve in one direction. To check this observation counts were made of the rotating colonies of *B. circulans*, *B. alvei*, and *B. sphaericus* var. *rotans*. The greatest proportion of colonies rotated in a counterclockwise direction, in a ratio close to 2:1 (table 1). There is no significant difference between the percentages for each species. It can be seen that the deviation from the expected 1:1 ratio is greater than would be expected by chance.

TABLE 1

Direction of rotation of colonies

	CLOCKWISE		COUNTERCLOCKWISE		S.E.%
	Number observed	%	Number observed	%	
B. circulans 715	278	33.3	559	66.7	±1.6
B. alvei 662	71	32.0	156	68.0	±3.1
B. sphaericus var. *rotans* 633	57	34.5	108	65.5	±3.7

The bullet type of colony usually takes a curved path in migration across the agar. These curves might be expected to correspond with rotating colonies, especially in the case of *B. alvei* and *B. sphaericus* var. *rotans*, because a large proportion of the rotating colonies are derived from bullet colonies. The curves taken by all the bullet colonies observed were recorded for the instant of observation. The figures obtained are shown in table 2. The deflection of bullet colonies was predominantly counterclockwise, and the proportions observed were not significantly different from the figures for the rotating colonies.

One strain of each species was observed for the data so far presented. To check the constancy within a species, the deflections of bullet colonies in seven strains of *B. alvei* were recorded (table 3). There is good correspondence between the figures for each strain, and the predominance of counterclockwise deflection is maintained. We do not possess enough authenticated strains of the other species to do a similar study, although three strains of *B. circulans* behaved in the same fashion.

By careful subculture of individual moving units, attempts were made to isolate strains showing pure clockwise or counterclockwise deflection. All such attempts failed, confirming the experience of Russ-Münzer (1938). The resulting isolates all showed the same counterclockwise-clockwise ratio of 2:1 as did the parent culture.

Direction of movement according to colony size. This was investigated in the case of rotating colonies of *B. circulans* and not further pursued. No significant

TABLE 2

Direction of deflection of "bullet" colonies

	CLOCKWISE		COUNTERCLOCKWISE		S.E.%
	Number observed	%	Number observed	%	
B. circulans 715	43	35.5	78	64.5	±4.35
B. alvei 662	50	32.5	104	68.5	±3.8
B. sphaericus var. *rotans* 663	55	34.6	104	65.4	±3.8

TABLE 3

Comparison of different strains of B. alvei: direction of deflection of "bullet" colonies

STRAIN	CLOCKWISE		COUNTERCLOCKWISE	
	Number observed	%	Number observed	%
662	50	32.5	104	68.5
127	55	34.3	105	65.7
179	52	33.3	104	66.7
408	61	37.9	100	62.1
343	52	32.5	108	67.5
551	59	34.1	114	65.9
552	56	34.3	107	65.7

differences were detected in the ratios of clockwise-counterclockwise rotation in the size groups 0.025 to 0.075 mm and 0.075 to 0.15 mm. They are compared in a contingency table (table 4) in which the two groups show virtually identical distributions. It may be concluded that colony size has no effect on the predominance of counterclockwise rotation.

The effect of position of the agar. It seemed possible that these movements might be affected by general external forces such as the rotation of the earth. Trials were made by maintaining the agar, after inoculation, in various positions relative to the earth's surface. No differences in the degree or characteristics of swarming were noted in any position. That the rotational field of the earth plays no part was shown by growing the organisms on duplicate plates, one oriented with the agar surface downwards and the other with the agar surface upwards. It is shown in table 5 that the direction taken by the rotating colonies of *B. circulans* is in relation to the agar surface and not to the surface of the earth.

A comparison with B. cereus var. *mycoides.* The colonies of *B. cereus* var. *mycoides* are rhizoid and spreading. At the margin of these colonies can be seen projecting filaments that may curve in either a clockwise or counterclockwise direction. The mechanism of spreading is probably different in this case from that described for swarming. The organisms are either weakly motile or non-motile in fluid media, and on solid media the extension seems to be due to elongation by growth of the very long, rigid filaments that are characteristic of the organism. Although the mechanisms may not be analogous, the filamentous extensions are curved and can be enumerated. Fourteen strains of *B. cereus* var. *mycoides* were examined, of which eight produced predominantly counterclockwise curves. The results of counts upon these strains are shown in table 6. It can be seen that the predominantly counterclockwise strains are remarkably constant and on average are very close to a 3:1 ratio. The predominantly clockwise strains show a little more variability and the mean ratio lies between 2:1 and 3:1.

TABLE 4

Contingency table comparing two size groups of rotating colonies of B. circulans

RANGE OF DIAMETER OF COLONY IN MM	NUMBER OF COLONIES	
	Clockwise	Counterclockwise
0.025–0.075	42 (41.3)	92 (92.2)
0.075–0.15	51 (51.2)	113 (112.8)

The expected values are in parentheses.
$\chi^2 = 0.00256$. $p = 0.96$.

TABLE 5

The effect of position of the agar on colony rotation

B. CIRCULANS 715	DIRECTION OF ROTATION			
	Clockwise		Counterclockwise	
	Number observed	%	Number observed	%
Grown agar surface upward..........	278	33.3	559	66.7
Grown agar surface downward.......	469	34.2	903	65.8

Directions are recorded as if looking at agar surface.

The phenomenon of elasticotaxis. This phenomenon has been described many times for *B. cereus* var. *mycoides* and was recently described for myxobacteria by

Stanier (1942) together with a neat method of demonstration. If a stress is put on the agar, the filaments of *B. cereus* var. *mycoides* extend along the lines of stress instead of following their usual curved path. Rectangular pieces of agar were cut out of an agar plate and draped over a glass rod in a Petri dish. Inoculation was made at a central point directly over the glass rod. The lines of stress run straight down from the glass rod to the point at which the sheet of agar touches the dish. The developing swarm was observed at frequent intervals. In the case of *B. alvei* and *B. sphaericus* var. *rotans*, which produce predominantly bullet colonies, there was good evidence, from the asymmetry of extension, that the swarming units tended to follow the lines of stress. In the case of *B. circulans*, which produces predominantly rotating colonies, the swarm extended in an even and regular fashion giving no definite evidence of elasticotaxis. In this case, although no counts were made, it was observed that both clockwise and counter-

TABLE 6

Curvature of terminal filaments of B. cereus var. mycoides

STRAIN	% CLOCKWISE	% COUNTER-CLOCKWISE	STRAIN	% CLOCKWISE	% COUNTER-CLOCKWISE
273	29	71	326	74	26
371	26	74	A1000	66	34
911	24	76	317	72	28
912	22	78	A967	73	27
936	23	77	A987	72	28
233	26	74	319	71	29
Mean.......	25	75	A966	64	36
			A306	62	38
			Mean.......	69.3	30.7

The total number observed for each strain was between 150 and 175.

clockwise rotating colonies were formed. The symmetry of swarm extension is not affected by gravity.

The degree of elasticotaxis shown by bullet colonies may be due to the structure of the colony. Except at the anterior end, most of the bacilli are arranged with their long axes in the direction of movement. The rotating colony is round and the long axes of the bacilli are oriented tangentially.

Since elasticotaxis may be due to orientation of the long polymeric molecules in the agar, attempt was made to get orientation by a different method. Agar was poured on a slightly tilted, cooled plate of glass so that solidification occurred during the flow. The method was extremely rough, but, in two out of ten trials, preparations were obtained on which *B. cereus* var. *mycoides* showed definite "elasticotaxis." With this result in mind, it was suspected that the time-honored rotatory movement given to plates after pouring might be affecting the movement of swarming units. However, comparison of counts made on plates that had been swirled in either direction, or not at all, showed that this made no change in the deflections observed for the bullet colonies of *B. alvei*.

DISCUSSION

From the morphological point of view there is no reason to expect polarity of the individual bacilli. The swarming species are all peritrichate and have no apparent constant asymmetry except a tendency to form excentric spores. We assume, for the time being, that individual cells swimming in a fluid have an unstable polarity that is constantly undergoing redistribution, possibly at random. In the swarming phenomenon we have aggregations of cells maintaining a remarkably stable polarity as a group, expressed as movement. From both direct and circumstantial evidence we conclude that swarming is an expression of motility under special conditions—in the thin fluid layer overlying the gel. Although the mechanism of aggregation into groups and the principle conferring polarity on that group are unknown, it would be expected that the units so formed would move at random in the two dimensions available to them.

Although a swarm covers an agar surface in a regular fashion from a point of origin, the evidence presented shows that the swarming units have an unexpected deviation from movement at random. This is expressed as a predominance of counterclockwise movement of rotating colonies and a predominance of counterclockwise curves taken by bullet colonies. Despite this deviation from the expected, swarms cover the surface of agar in a regular fashion because the migrating unit can start from the point of origin in any direction. The correspondence between rotating and bullet colonies might be expected since in many species the bullet colonies often take a tightening spiral path and become rotating colonies. In *B. circulans*, however, many rotating colonies are initiated by rotation in a small mass of actively growing cells without the intermediation of a bullet type of colony.

Since the position of the agar surface relative to gravity does not alter the phenomenon, the evidence is against, but does not exclude, the cause of this phenomenon being an external influence. From a consideration of elasticotaxis it is possible that the physical state of the agar conditions the effect. Although the bullet type of colony may be directed to some extent along lines of stress in the agar, the rotating colonies are not obviously affected. If the curvature of filaments of *B. cereus* var. *mycoides* is analogous to the rotation of other *Bacillus* species, then the development of both clockwise and counterclockwise strains on a single agar plate would be contrary to such a hypothesis.

There remains the possibility that these peculiarities of action are due to some inherent characteristic of individuals in the bacterial population. There is some basis for this hypothesis in the case of *B. cereus* var. *mycoides*. Gause (1939) suggested that the dextral form is a mutant of the more commonly occurring sinistral form in which the inversion of the growth of filaments is associated with the presence of D-isomers in the protoplasm. He supported his hypothesis to some extent by detecting in the dextral form an enzyme splitting the unnatural D-peptides (Gause, 1942). Alpatov and Nastyukova (1947) determined the relative toxicity of the optical isomers of mepacrine upon dextral and sinistral strains of *B. cereus* var. *mycoides*. The dextral form is inhibited to a greater extent by D-mepacrine, and the sinistral form is inhibited to a greater extent by

L-mepacrine. It must be emphasized that it is dangerous to compare too closely the activities of *B. cereus* var. *mycoides* and the swarming species of *Bacillus*. This is not only because the mechanisms of extension may be different but also because swarming strains have not been found that produce predominantly clockwise (dextral) deflections. However, the association is close enough—being related within the same genus, and the deflections being of the same general order—to warrant further study on biochemical lines and search for strains of *B. circulans*, *B. alvei*, and *B. sphaericus* that go contrary to the counterclockwise predominance demonstrated in this paper.

If the bacterial population is not homogeneous and consists of two variant types, of which one predominates slightly over the other determining the direction of deflection according to relative numbers, it would be possible to fit a hypothesis to the observed effect. In this case the ratios of deflection would have to vary, in the direction of the predominant type, in proportion to the number of individuals in the group. However, this hypothesis is upset by the close correspondence of the ratios observed for the rotating colonies of *B. circulans* grouped according to the size of colony.

It is not known whether such peculiarities may be detected in the motility of individual organisms. However, it could be suspected because individual filaments of *B. alvei* may be observed, on insufficiently dried agar, to move in a circular path like a toy train on a circular track. The fact remains that in swarming, which is a by-product of motility, the curved path traced by bullet colonies and the direction in which a colony rotates are not determined by chance.

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SUMMARY

The figures presented show that the swarming units of *Bacillus circulans*, *Bacillus alvei*, and *Bacillus sphaericus* var. *rotans* do not move at random. With each species the majority of rotating colonies turn in a counterclockwise direction in a ratio (counterclockwise:clockwise) of 2:1. The same ratio obtains when the curves taken by the migrating bullet-shaped colonies are enumerated. This ratio is remarkably constant and would not be expected by chance.

Evidence is given to show that the phenomenon is not likely to be due to external influences such as the rotational field of the earth or to a slight predominance of a variant in the bacterial population.

Comparison is made with the curving tendency of the terminal filaments around colonies of *Bacillus cereus* var. *mycoides*. In this species strains are found showing either predominantly clockwise or counterclockwise curves. Examination of a number of strains of each tendency shows that the ratios obtained are

close to 3:1 in all cases. The mechanism of colony extension is probably different from that of the other species. Strains of *B. circulans*, *B. alvei*, or *B. sphaericus* showing predominantly clockwise motion have not been found.

It is considered that swarming is an expression of motility in special restricted circumstances. If this is true it may be that the tendency to move in regular curves is an inherent property of organisms that is not detected in studies of motility.

REFERENCES

ALPATOV, V. V., AND NASTYUKOVA, O. K. 1947 The specific action of optical isomers of mepacrine upon dextral and sinistral strains of *Bacillus mycoides* Flugge. Am. Rev. Soviet Med., **5**, 28–31.

ASHESHOV, I. N. 1941 Papain digest media and standardisation of media in general. Can. J. Pub. Health, **32**, 468–471.

BOLTJES, T. Y. K. 1948 Function and arrangement of bacterial flagella. J. Path. Bact., **60**, 275–287.

CLARK, F. E. 1939 Non-motile variants of *Bacillus alvei*. J. Bact., **38**, 491–497.

GAUSE, G. F. 1939 Some physiological properties of dextral and sinistral forms in *Bacillus mycoides* Flugge. Biol. Bull., **76**, 448–465.

GAUSE, G. F. 1942 Biokhimia, **7**, 1. (Original not seen; cited by Alpatov and Nastyukova.)

HENRIKSEN, S. D., AND SVENDSEN, M. 1946 Rotating and wandering colonies in a strain of genus *Bacillus*. Acta Path. Microbiol. Scand., **23**, 387–394.

MURRAY, R. G. E., AND ELDER, R. H. 1948 The motile colonies of bacterial swarms. Proc. Soc. Am. Bact., **1**, 4.

MURRAY, R. G. E., AND ELDER, R. H. 1949 Evidence that bacterial motility may not be at random. Can. J. Pub. Health, **40**, 35.

MUTO, T. 1904 Ein eigentumlicher *Bacillus*, welcher sich Schneckenartig bewegende Kolonien bildet (*B. helixoides*). Zentr. Bakt. Parasitenk., I, Orig., **37**, 321–325.

ØRSKOV, J. 1947 Method for the demonstration of bacterial flagellum activity. Acta Path. Microbiol. Scand. **24**, 181–183.

PIJPER, A. 1946 Shape and motility of bacteria. J. Path. Bact., **58**, 325–342.

ROBERTS, J. L. 1935 A new species of the genus *Bacillus* exhibiting mobile colonies on the surface of nutrient agar. J. Bact., **29**, 229–238.

ROSZDESTVENSKY, V. S., AND MEDVINSKA, L. 1938 Motility of colonies of the new species of *Bacillus migrans*. Microbiology (Moskva), **7**, 137–141 (pt. 2). English abstract p. 142.

RUSS-MÜNZER, A. 1938 Ueber einen eigenartigen Bewegungstypus schwarmender Bakterien. Zentr. Bakt. Parasitenk., I, Orig., **142**, 175–190.

SHINN, L. E. 1938 A cinematographic analysis of the motion of colonies of *B. alvei*. J. Bact., **36**, 419–422.

SHINN, L. E. 1940 The motion of colonies of *Bacillus alvei* as shown by lapse-time cinematography. J. Bact., **39**, 22.

SMITH, N. R., AND CLARK, F. E. 1938 Motile colonies of *Bacillus alvei* and other bacteria. J. Bact., **35**, 59–60.

STANIER, R. Y. 1942 A note on elasticotaxis in myxobacteria. J. Bact., **44**, 405–412.

TURNER, A. W., AND EALES, C. E. 1941*a* An aerobic, sporulating *Bacillus* that forms rotating and migrating colonies. Australian J. Exptl. Biol. Med. Sci., **19**, 161–166.

TURNER, A. W., AND EALES, C. E. 1941*b* Motile daughter colonies in the *Cl. oedematiens* groups and some other clostridia (*Cl. botulinum* C, *Cl. tetani*, *Cl. septicum*). Australian J. Exptl. Biol. Med. Sci., **19**, 167–174.